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Effects of endothelin-1 on renal function in humans: Implications for physiology and pathophysiology

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Effects of endothelin-1 on renal function in humans: Implications for physiology and pathophysiology. Elevated levels of the vasocontrictor peptide endothelin-1 have been demonstrated in various pathological conditions that are characterized by sodium retention and/or renal vasoconstriction, such as heart failure, hepatorenal syndrome, renal failure and during administration of cyclosporin and radiocontrast. In the present study we studied in seven healthy subjects the renal and endocrine effects of systemic administration of endothelin-1 (0.5, 1.0 and 2.5 ng/kg/min). During endothelin-1 infusion plasma levels rose from 3.2 ± 0.5 to respectively 5.0 ± 0.8, 6.2 ± 0.5 and 8.5 ± 1.1 pmol/liter, values that can also be observed in physiological and pathological conditions. Infusion of low dosages of endothelin-1, that result in a twofold increase in plasma levels, decreased sodium excretion by 36%, without a significant effect on systemic and renal hemodynamics. Infusion of 2.5 ng/kg/min of endothelin-1 further enhanced sodium retention and, in addition, increased renal vascular resistance by 37%. Blood pressure did not change significantly. Pretreatment with the calcium channel blocker nifedipine caused renal vasodilation, which compensated for the renal vasocontriction by endothelin-1 and prevented sodium retention. Apparently, endothelin-1 participates in volume homeostasis in humans, whereas pathophysiologiocal concentrations can contribute to renal vasoconstriction and sodium retention. Calcium channel blockers may protect against these effects of endothelin-1.

Elevated levels of the vasoconstrictor peptide endothelin-1 have been demonstrated in many pathological conditions [1, 2]. Local factors such as thrombin, fluid-mechanical shear stress and tumor necrosis factor can enhance endothelin synthesis by the vascular endothelium [1]. These factors may contribute to the elevated levels of endothelin found in conditions with generalized endothelial damage such as atherosclerosis [3] and during cyclosporin administration [4]. Elevated plasma levels of endothelin have also been found in situations where effective circulating volume is decreased, such as heart failure [5] and hepatorenal syndrome [6], and in renal failure [7, 8].

It is possible that increased plasma levels of endothelin-1 contribute to the renal vasoconstriction, decreased GFR, and sodium retention that occur in these pathological conditions. However, the effects of systemic infusion of endothelin-1 in humans have hardly been studied [9, 10], and have thus far focused on the effects on blood pressure and regional clearance of endothelin. In the present study, we therefore investigated the effects of increasing endothelin-1 dosages on renal hemodynamics and excretory function and on endocrine function in men up to plasma levels of ± 10 pmol/liter, levels found during the pathological conditions mentioned above. At present no information is available whether the renal effects of endothelin-1 can be prevented in humans. Because recruitment of voltage-dependend Ca^{2+}-channels probably contributes to the vasoconstrictive effect of endothelin [11], we also investigated whether blockade of these channels with the dihydropyridine nifedipine can modulate the renal effects of endothelin-1 in humans, and in this way could be used as a treatment modality.

Methods

Studies were carried out in seven healthy volunteers (coworkers from the department of nephrology). Six were males, one was female; their ages ranged from 27 to 33 years. The protocol was approved by the University Hospital Ethical Committee for study in humans (no. 93.93).

All subjects underwent two clearance studies (see below). One study served as a time control study. During the other study endothelin was infused at incremental dosages (respectively 0.5, 1.0 and 2.5 ng/kg/min). Four subjects underwent an additional clearance study, during which the same quantities of endothelin were infused in the presence of nifedipine. For this purpose nifedipine infusion(priming dose 0.01 mg/kg, maintenance infusion 0.01 mg/kg/hr; Bayer BV, Mijdrecht, The Netherlands) was started at 9 a.m. on the morning of the clearance study, and continued throughout the study.

The subjects received a diet containing 200 mmol sodium and 100 mmol potassium per day. Adherence to the diet was controlled by 24-hour urine collections. Lithium carbonate (400 mg) was taken at 10 p.m. on the fourth day, and the subjects refrained from food thereafter. On the morning of the fifth day the subjects underwent a clearance study. At 9 a.m. a priming dose of a solution containing 10% inulin, to measure glomerular filtration rate (GFR), and 2.5% para-aminohippuric acid (PAH), to measure estimated renal plasma flow (ERPF), was administered, followed by continuous infusion of this solution throughout the remainder of the study. Maximal water diuresis was induced by an oral water load of 25 ml/kg body weight, and was maintained by drinking amounts of water matching urinary output. After at least one hour equilibration, and only when urine osmolality was 70

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mOsm/kg or less, two 20-minute urine baseline collections were obtained by spontaneous voiding. Blood specimens were drawn at the midpoint of each collection period from the contralateral forearm. Thereafter endothelin-1 infusion was started via a separate antecubital vein. Endothelin-1 (Peptide Institute Inc, Scientific Marketing Associates, Herst, UK) was dissolved in haemaccel and administered for 40 minutes in a dosage of 0.5 ng/kg/min, followed by a 40 minute infusion in a dosage of 1.0 ng/kg/min and a further 40 minute infusion with 2.5 ng/kg/min. The infusion period was followed by a recovery period of 120 minutes. Urine and blood sampling was continued at 20 minute intervals throughout the whole study. Samples for determination of plasma endothelin were obtained before endothelin-1 infusion, and every 40 minutes during the endothelin-1 infusion and recovery. Plasma renin (PRA), aldosterone and ANP were measured in blood samples drawn before, following 120 minutes of endothelin infusion and after 120 minutes of recovery.

Blood pressure and heart rate were recorded at five minute intervals during the clearance studies using an automatic oscillometer device (Omega 2000, Invivo Research Laboratory Inc. Tulsa, Oklahoma, USA). Mean arterial blood pressure was calculated as the sum of one-third of the systolic pressure and two-thirds of the diastolic pressure. Blood and urine samples were analysed for sodium and potassium by flame photometry, lithium (Perkin-Elmer 3030 Atomic Absorption Spectrophotometer) and osmolality by freezing point depression. Inulin was hydrolyzed to fructose and measured by photometry with indolacetic acid [12], and PAH was determined photometrically by a chromaldehyde reaction [13]. PRA, aldosterone and ANP were determined by radioimmunoassay, as described previously [14]. Blood samples for determination of endothelin-1 were collected in prechilled potassium EDTA containing tubes and centrifuged at 4°C. The plasma samples were stored at −70°C until the assay. Endothelin was extracted from plasma by solid phase extraction with 6-ml octadecyl columns (Baker, Phillipsburg, New Jersey, USA). After conditioning of the columns with methanol and deionized water, 2 ml of plasma acidified with 4 ml 0.5% trifluoroacetic acid (TFA) was applied to the columns and extracted, followed by washing with 6 ml 0.5% TFA and 6 ml deionized water, and elution with 2 ml 0.1% TFA in methanol: water 9:1 vol/vol. The eluate was dried under nitrogen at 40°C, the residue was dissolved in 250 μl assay buffer, and 100 μl aliquots were analyzed in duplicate. The recovery of endothelin throughout the procedure was 75%. Endothelin-1 was determined with radioimmunoassay kits (Peninsula Laboratories Europe, Merseyside, UK). Cross-reactivities of endothelin-2 and endothelin-3 are 7%, and of pro-endothelin-1 17%. The reported concentrations (pmol/liter) are corrected for procedural losses. Sensitivity of the assay was 0.3 fmol per assay tube; within-assay and between-assay coefficients of variation were 9% and 13% at a level of 3.6 pmol/liter, respectively.

Calculations and statistics

Reported values represent means ± standard error. PRA and aldosterone were analyzed after logarithmic transformation. Renal blood flow was calculated by dividing ERPF by (1-packed cell volume), and renal vascular resistance was calculated by dividing arterial pressure by renal blood flow. Statistical analysis was performed using two-way analysis of variance of a randomized block design to compare the endothelin infusion to time control. Plasma endothelin levels were analyzed with one-way analysis of variance, as plasma levels were less frequently sampled during the time-control study. If variance ratios reached statistical significance, the differences between the means were analyzed with the Studentized Newman-Keuls test for $P < 0.05$ and $P < 0.01$.

Results

Twenty-four-hour urine sodium excretions were 210 ± 22, 190 ± 31 and 194 ± 44 mmol on the days before the time-control study, endothelin infusion study and endothelin with nifedipine infusion study, respectively. Endothelin-1 infusion was well tolerated in the dosages used, and no side effects were observed.

Effects of endothelin-1 on electrolyte excretion

Sodium excretion decreased progressively during endothelin-1 infusion from a baseline value of 196 ± 33 μmol/min to 79 ± 13 μmol/min during the highest dosage of endothelin. After cessation of the endothelin-1 infusion sodium excretion did not return to levels observed during the time control study (Fig. 1). Fractional sodium excretion (that is, corrected for differences in filtered load) decreased parallel to sodium excretion from a baseline value of 1.24 ± 0.2 to 0.56 ± 0.1% during 2.5 ng/kg/min endothelin-1 infusion ($P < 0.01$, compared to time control). The decrease in sodium excretion was accompanied by a progressive decrease in fractional excretion of lithium (24.0 ± 2.3 to 18.9 ± 1.7%, $P < 0.01$) and maximal urine flow (14 ± 1 to 8 ± 1 ml/min, $P < 0.01$), whereas urine osmolality did not change (66 ± 4 to 64 ± 4 mOsm/kg). Potassium excretion during endothelin-1 infusion did not differ from time-control observations.

Effects of endothelin-1 on hemodynamics

Endothelin-1 did not result in a significant increase in blood pressure, although a tendency towards a higher blood pressure was noted during the highest dose of endothelin (91 ± 2 to 94 ± 2 mm Hg, not significant). Heart rate did not change (57 ± 2 to 55 ± 2 beats/min). GFR did not change significantly until the highest dose of endothelin, during which it decreased from 121 ± 8 to 109 ± 9 ml/min ($P < 0.05$). Similarly, ERPF did not change significantly except during the highest endothelin infusion (Fig. 2). The decrease in renal plasma flow at this dosage proportionally exceeded the decrease in GFR, as reflected by an increase in filtration fraction from 20.6 ± 1.5 to 24.5 ± 2.0% ($P < 0.01$). As a result of these hemodynamic changes, calculated renal vascular resistance increased during the 2.5 ng/kg/min endothelin infusion from 82 ± 3 to 112 ± 5 mm Hg · min/liter ($P < 0.01$), but was not significantly different from time-control measurements during the other dosages of endothelin-1.

Effects of endothelin-1 on hormones

Infusion of endothelin-1 resulted in a threefold increase of plasma endothelin (Fig. 3). Plasma renin activity, plasma aldosterone and ANP levels did not change significantly during endothelin infusion (from 164 ± 59 to 131 ± 62 fmol · liter/second, 242 ± 57 to 217 ± 61 pmol/liter and from 10 ± 2 to 12 ± 4 pmol/liter, respectively).

Endothelin-1 infusion in the presence of nifedipine

Baseline sodium excretion in the subjects who participated in both the endothelin-1 infusion studies was 214 ± 62 μmol/min during the control endothelin infusion and 238 ± 53 μmol/min during the nifedipine study. Baseline blood pressure was similar

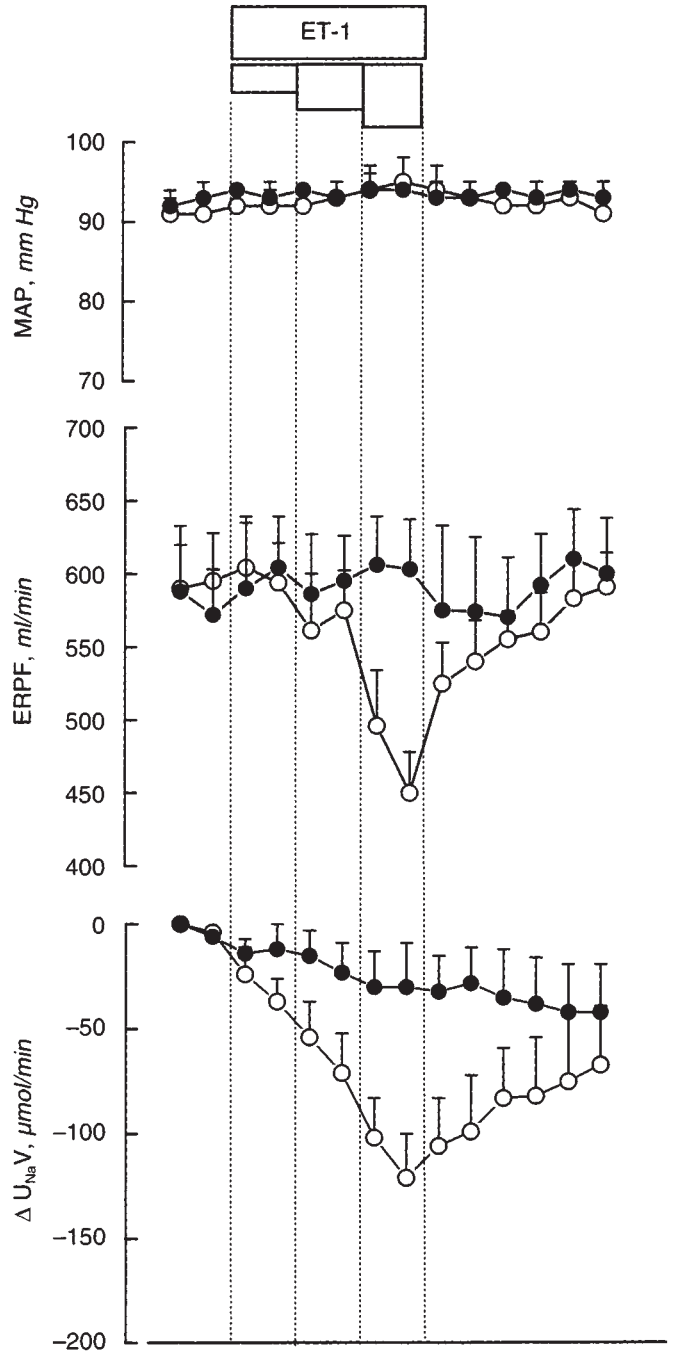

Fig. 1. *Changes in sodium excretion (Δ $U_{Na}V$, lower panel), renal plasma flow (ERPF, middle panel) and mean arterial blood pressure (upper panel) during endothelin infusion (○) versus time-control (●) in 7 subjects.* Values are means ± SEM. Each values represents the mean of 20 minutes. Endothelin-1 (ET-1) was given at incremental dosages of 0.5, 1.0 and 2.5 ng/kg/min for 40 minute periods (indicated by the boxes), followed by 2-hour recovery measurements.

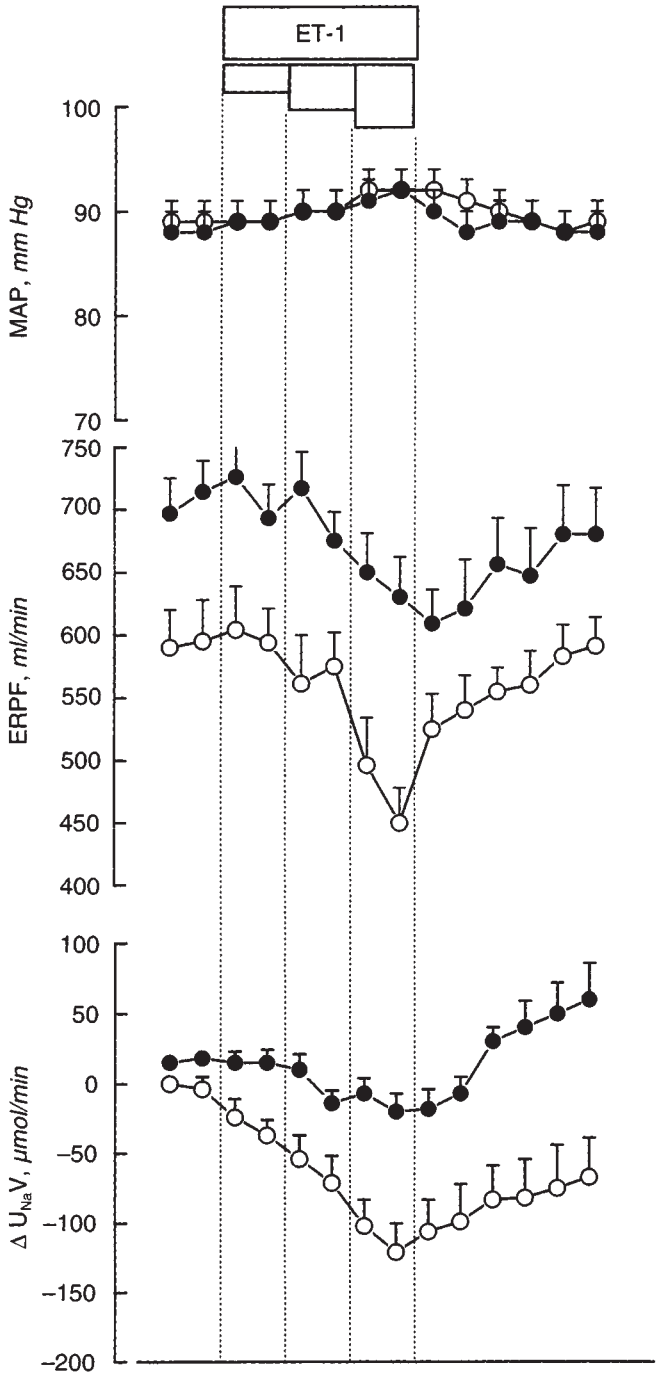

Fig. 2. *Changes in sodium excretion (Δ $U_{Na}V$, lower panel), renal plasma flow (ERPF, middle panel) and mean arterial blood pressure (upper panel) during endothelin infusion (○) and endothelin infusion after nifedipine pretreatment (●) in 4 subjects.* Values are means ± SEM. Each values represents the mean of 20 minutes. Endothelin-1 (ET-1) was given at similar dosages as in Figure 1.

between the control endothelin infusion and the nifedipine study (89 ± 2 and 88 ± 2 mm Hg, respectively). In the nifedipine study there was a trend towards a lower sodium excretion during endothelin infusion when compared to pre-infusion values, which was caused by a slight antinatriuresis in two subjects. However, sodium excretion exceeded that during time control in all subjects during the whole infusion period. Cessation of the endothelin infusion was followed by an increase in sodium excretion in all

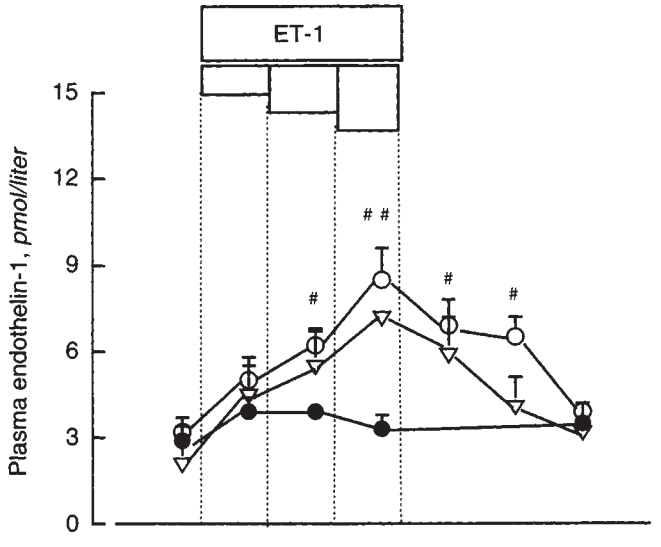

Fig. 3. *Plasma endothelin-1 levels during endothelin infusion (○), time-control (●) in 7 subjects and during endothelin infusion after nifedipine treatment (▽) in 4 subjects.* Values are means ± SEM. Each value represents the mean of 40 minutes. # $P < 0.05$, ## $P < 0.01$ compared to baseline for the endothelin study. Endothelin-1 (ET-1) was given at similar dosages as in Figure 1.

subjects. Baseline ERPF was increased and renal vascular resistance decreased during nifedipine pretreatment in all subjects, when compared to the baseline value in the endothelin infusion study (717 ± 7 ml/min vs. 595 ± 33 ml/min and 69 ± 2 vs. 81 ± 2 mm Hg · min/liter, respectively). Renal plasma flow fell during endothelin infusion in the nifedipine study (Fig. 2) and renal vascular resistance increased from 69 ± 2 to 79 ± 3 mm Hg · min/liter. However, ERPF exceeded baseline levels of the endothelin study during all clearance periods, while renal vascular resistance did not increase above time-control levels. GFR did not change during endothelin-1 infusion in the nifedipine study, and filtration fraction increased from 18.4 ± 1.0 to 20.0 ± 0.5% in all subjects. Increments in plasma endothelin were comparable between the two studies (Fig. 3).

Discussion

The present study shows that infusion of endothelin-1 in low dosages in humans causes renal sodium retention, whereas a higher dosage of endothelin causes strong sodium retention and, in addition, renal vasoconstriction. The lowest antinatriuretic dosages doubled plasma endothelin-1 concentrations. The highest dosage increased plasma endothelin-1 threefold, to concentrations seen in pathophysiological conditions, such as heart failure, hepatorenal syndrome and renal failure [5–8]. Therefore, our study is the first to show functional information that physiological concentrations of endothelin-1, such as that observed during upright tilt [15], participate in volume homeostasis, whereas pathophysiological concentrations can contribute to renal vasoconstriction and sodium retention. Finally, our data also demonstrate that nifedipine prevents the antinatriuresis by endothelin-1, and causes renal vasorelaxation sufficient to compensate for the endothelin-1-induced renal vasoconstriction.

At the lower dosages of endothelin-1 sodium excretion decreased in the absence of significant changes in filtration, whereas after discontinuation of endothelin-1 renal hemodynamics rapidly normalized, while sodium retention continued (Fig. 1). This indicates that endothelin-1 may directly stimulate tubular sodium reabsorption. The decrease in sodium excretion was accompanied by a fall in fractional excretion of lithium and maximal urine flow. With appropriate reserve, this could indicate an increase in proximal tubule sodium reabsorption and/or increased sodium reabsorption in the thin segments of Henle's loop [16]. The precise mechanism of this sodium retention is unclear at the moment. In animals studies no receptors for ET could be identified in the proximal nephron [17]. This may indicate that the endothelin-receptor distribution may differ among species. Alternatively, the stimulatory effects on sodium reabsorption in the proximal nephron are secondary to renal hemodynamic changes, such as a redistribution of renal blood flow towards the cortex, which increases sodium reabsorption in medullary nephron populations but does not alter whole kidney blood flow. The marked antinatriuretic effect of endothelin-1 in humans is in contrast with animal studies. Most of these studies demonstrated a natriuretic effect of low dose systemic administration of endothelin-1 despite a fall in GFR and renal blood flow [18–20]. This natriuresis may be due to stimulation of ANP [21, 22] or pressure-natriuresis secondary to a higher blood pressure during endothelin infusion. Studies in the isolated perfused kidney have also shown an increase in sodium excretion after endothelin-1 administration [23]. Systemic infusions with high doses of endothelin-1 in dogs decreased sodium excretion because of reduction in filtered load and/or renin-angiotensin stimulation [24, 25]. It should be noticed, however, that in the present study sodium excretion decreased during the lower dosages of endothelin-1 in the absence of a change in filtered load or in activity of the renin-angiotensin system.

In contrast to the antinatriuretic effect of endothelin-1 infusion, which already appeared during the lower dosages of endothelin, renal vasoconstriction in our study occurred only at the highest dosage of endothelin. At this dose renal vascular resistance increased by approximately 37%. Similarly, GFR decreased only at the highest dose of endothelin-1. Importantly, blood pressure did not change at this endothelin-1 dosage. This suggests that elevations of endothelin-1 in humans, unlike other vasopressor systems such as angiotensin II, do not contribute to acute blood pressure regulation. Of course, it is very well possible that the renal effects of endothelin-1 contribute to long-term blood pressure regulation.

At least two intracellular signaling pathways for the hemodynamic effects of endothelin have been identified thus far. Endothelin increases membrane diacylglycerol, thus activating protein kinase C, and endothelin induces phosphatidylinositol breakdown, which results in release of Ca^{2+} from intracellular stores [26, 27]. The increase in inositol triphosphate and/or calcium activates voltage-dependent calcium channels, thereby also mobilizing extracellular calcium. The latter pathway suggests that blockade of these voltage-dependent Ca^{2+} channels may also modulate the effects of endothelin. However, the

observed effects of Ca^{2+} antagonists on vasoconstriction induced by endothelin-1 vary greatly, and seem to depend on the blood vessels and species studied, as well as the experimental conditions, such as endothelin dosage [2]. The Ca^{2+} antagonist manidipine was capable of near-total reversal of the effects of endothelin on blood pressure and renal blood flow in rats [28], while other studies in intact animals failed to show effects of Ca^{2+} antagonists on the renal vasoconstrictive properties of endothelin [29, 30], or could only demonstrate an attenuation at lower dosages of endothelin [31, 32]. The present study shows that pretreatment with nifedipine at a dosage that does not affect blood pressure can compensate for both the renal vasoconstrictive and antinatriuretic effects of endothelin. Some effect of endothelin still can be observed, which is not surprising in view of the existence of other intracellular signaling pathways. Alternatively, one has to consider the possibility that the attenuation of the effects of endothelin during nifedipine infusion was aspecific by resetting renal blood flow and sodium excretion to levels higher than baseline. Nevertheless, irrespective of the mechanism, renal perfusion and sodium excretion never fell below control levels. This observation suggests a renal protective effect of Ca^{2+} antagonists in pathological conditions, which are associated with elevation of plasma endothelin such as contrast administration [33], post-ischemic renal failure [7] and administration of cyclosporin [4]. It also coincides with some recent observations that Ca^{2+} antagonists decrease renal vasoconstriction and improve renal transplant survival in renal transplant recipients treated with cycloporin [34, 35].

The present study demonstrates a role for endothelin in physiological volume homeostasis, as well as in pathological volume derangements and during loss of renal function in men by inducing renal vasoconstriction and sodium retention. Calcium channel blockers may prevent these effects of endothelin.

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